A PHYLOGENY OF *ARCTOSTAPHYLOS* (ERICACEAE) INFERRED FROM NUCLEAR RIBOSOMAL ITS SEQUENCES

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ABSTRACT

A phylogeny of *Arctostaphylos* (Ericaceae) was estimated using nuclear ribosomal ITS sequence data for 98 species, of which 83 were unique taxa and 15 were multiple samples of geographically widespread or morphologically variable species (*A. uva-ursi*, *A. patula*, *A. pungens*, *A. manzanita*, *A. glandulosa*, and *A. tomentosa*). Forty-four ingroup taxa shared an identical sequence. The remaining species showed pairwise substitution rates of 2.4% or less, and 17 taxa had sequences with polymorphic positions. Maximum parsimony analysis recovered a large polytomy and three small clades. Analysis of a reduced data set with the 17 polymorphic taxa removed recovered a two-clade topology with some additional resolution within each clade. Four taxa showed patterns of sequence nucleotide additivity suggesting interspecific hybrid origin. A proposed hypothesis of higher rates of nucleotide substitution in species that are obligate seeders vs. those that are facultative sprouters could not be tested due to shared sequences. Low interspecific sequence variability in the nuclear ribosomal ITS region in *Arctostaphylos* may be due to several factors, including recent diversification of the genus in California, long generation time between sexual reproduction events, and rapid concerted evolution of nuclear ribosomal ITS arrays in both diploids and tetraploids.

KEY WORDS: *Arctostaphylos*, generation time, interspecific hybridization, molecular phylogeny, nuclear ribosomal ITS, polyploidy, superimposed nucleotide additivity patterns.

RESUMEN

Una filogenia de *Arctostaphylos* (Ericaceae) se estableció utilizando secuencias del ADN nuclear ribosómico ITS de 98 especies de las cuales seis son de distribución geográfica amplia o morfológicamente variables están representadas por más de una accesión (*A. uva-ursi*, *A. patula*, *A. pungens*, *A. manzanita*, *A. glandulosa*, and *A. tomentosa*). Cuarenta y cuatro taxones comparten una secuencia idéntica. Las otras especies muestran una tasa de sustitución nucleotídica de 2.4% o menos, y en 17 taxones bases polimórficas. El análisis de máxima parsimonia conduce a una gran politomía, sin embargo eliminando los 17 taxones polimórficos, la topología del árbol nuevamente obtenido evidencia dos clados y algunos subclados. Cuatro taxones muestran patrones de secuencia de aditividad nucleotídica sugiriendo un origen híbrido interespecífico. La similitud de las secuencias de las especies que se reproducen estrictamente por semillas vs las que se reproducen por rebrotes y a la vez por semillas no permite confirmar la hipótesis de mayor tasa de sustitución nucleotídica en las primeras. La baja variabilidad interespecífica que muestra el ITS en *Arctostaphylos* puede deberse a varios factores, como la reciente diversificación del género en California, el largo tiempo de generación entre los eventos de reproducción sexual, y la evolución concertada rápida del ITS en las populaciones que han experimentado eventos de hibridación en ambos niveles diploides y tetraploides.

INTRODUCTION

Arctostaphylos Adans. (Ericaceae) is composed of ca. 108 taxa and is the most species-rich genus of subfamily Arbutoideae Nied. (Wells 2000). Except for *Arctostaphylos uva-ursi* (L.) Spreng.—which has a circumboreal distribution—the remaining species are confined to western North America and Mexico. In California, species of *Arctostaphylos* occur in a variety of plant communities, including chaparral, montane scrub, mixed-coniferous forests, serpentine barrens, and oak savannas. At low latitudes, *Arctostaphylos uva-ursi* occurs in montane regions (e.g., Rockies, Appalachians, Alps, Himalayas) and in coastal pine barrens and

dunes (e.g., New Jersey and Michigan, USA). *A. uva-ursi* subsp. *cratericola* (Donn.Sm.) P.V.Wells represents a disjunct population in the volcanic highlands of Guatemala.

Arctostaphylos is placed in subfamily Arbutoideae with five other genera: *Arctous* Nied., *Arbutus* L., *Comarostaphylis* Zucc., *Ornithostaphylos* Small, and *Xylococcus* Nutt. (Diggs & Breckon 1981). Molecular and morphological phylogenetic studies in the Ericaceae place the Arbutoideae between the basalmost Monotropoideae and the rest of the family (Kron et al. 2002). Markos et al. (1998) presented phylogenetic trees for 16 species of *Arctostaphylos* inferred from nuclear ribosomal internally transcribed spacer (nrITS) and 28S nuclear ribosomal sequences. The two-clade topology recovered in their trees was not congruent with the two subgenera of Wells (2000) nor did it support the monophyly of the *A. hookeri* G.Don species complex in section *Pungens* (Markos et al. 1998). Boykin et al. (2005) presented a nrITS phylogeny of 38 taxa that also recovered a two-clade topology. Further, these two-clade phylogenies are congruent (with respect to species composition) with a phenogram constructed from flavonoid data for 20 species of *Arctostaphylos* (Denford 1981).

The center of diversity for *Arctostaphylos* is centered about San Francisco, CA in the California Floristic Province. The coastal areas and Coast Ranges of California, from Mendocino County to Santa Barbara County, are particularly rich in taxa (Markos et al. 1998). *Arctostaphylos* has a high percentage of endemic taxa, many of which are edaphic specialists (Wells 2000). Nearly half of all California taxa are considered rare, threatened, or endangered (CNPS 2009). The diversity of modern taxa may be the result of a radiation of *Arctostaphylos* since the end of the Miocene (5.3 Ma) (Edwards 2004). Factors contributing to the diversification of the genus include local adaptation to California's diverse substrates, an increase in fire frequency at the end of the Pliocene, the onset of a Mediterranean climate, and the contemporaneous expansion of chaparral into forested areas (Raven & Axelrod 1978; Axelrod 1989; Edwards 2004).

Hybridization and polyploidization are two processes driving speciation in *Arctostaphylos*. The base chromosome number in *Arctostaphylos* is $x = 13$, with most species being either diploid ($2n = 26$) or tetraploid ($2n = 52$). Schierenbeck et al. (1992) provide morphological and cytological evidence for the allopolyploid origin of *A. mewukka* Merriam. Many other taxa have been proposed as arising via homoploid hybrid speciation (e.g., Dobzhansky 1953; Gottlieb 1968; Schmid et al. 1968; Kruckeberg 1977; Knight 1985; Keeley & Massihi 1994; Parker & Vasey 2004). For a group of 46 edaphic specialists or endemic taxa, Wells (2000) proposed a hybrid origin for 37. Differing ploidy levels observed in *Arctostaphylos uva-ursi* subsp. *coactilis* Fernald & J.F.Macbr. ($2n = 26$, 39, 52 and 78) suggests recent and recurring polyploidization, which is common in arctic plants (Rosatti 1981; Brochmann et al. 2004).

Morphological intermediacy is not always a reliable indicator of hybrid origin (e.g., Rieseberg 1995)—particularly in *Arctostaphylos*. It is likely that introgression and polyploidization in *Arctostaphylos* has lead to misleading phylogenetic inferences and taxonomic classifications that are based on morphological characters. In perennial species of *Paeonia* L. and *Sidalcea* A.Gray, the bi-parentally inherited, nrITS region has been used successfully to corroborate parentage of allopolyploid taxa (Sang et al. 1995; Whittall et al. 2000). In angiosperms, direct sequencing of the maternally inherited chloroplast may provide additional evidence of hybrid origin by identifying the maternal parent (Koontz et al. 2004). In *Arctostaphylos*, Wahlert (2005) sequenced five chloroplast regions (*trnL* intron and the intergenic spacers *atpB-rbcL*, *trnL-trnF*, *trnS-trnG* and *trnH-psbA*) for 12 morphologically divergent and geographically distant species. The resulting combined alignment was 1,836 base pairs (bp) long and had just three parsimony informative positions. Thus, direct sequencing of the chloroplast in *Arctostaphylos* could not be used to infer relationships or to confirm maternal parentage in hybrids species.

Different life history strategies in *Arctostaphylos* appear to influence the rate of speciation. In California's fire-prone chaparral communities, *Arctostaphylos* and *Ceanothus* L. (Rhamnaceae) are the only genera of sclerophyllous shrubs containing species that have evolved an obligate seeding mode of reproduction (Wells 1969). In these genera, in about two thirds of the species, adults are killed in response to fire and populations recover by recruitment from seed banks. Most chaparral shrubs re-sprout after a stand-replacing fire, but

in species of *Arctostaphylos* and *Ceanothus*, seeds are stored in a persistent soil seed bank from which they germinate only after fire (Parker and Kelly 1990). Therefore, each post-fire generation of obligate seeders is a product of sexual reproduction, whereas facultative sprouters regenerate primarily from vegetative re-sprouts (but also to a lesser degree from seed) (Keeley & Keeley 1981). A higher rate of morphological diversification and local endemism for seeders is supported by the fact that ca. 70% of *Arctostaphylos* taxa are obligate seeders (Wells 1969). Generation-time theory predicts that taxa with shorter generation times will have higher rates of molecular evolution in neutral DNA regions compared to taxa with longer generation times (e.g., Andreasen & Baldwin 2001). For that reason, it is hypothesized that in *Arctostaphylos*, there will be a higher rate of interspecific ITS sequence variability among obligate seeders compared to facultative sprouters.

The primary goal of this study was to estimate the phylogeny of *Arctostaphylos* with extensive taxon sampling using nrITS sequences. Increased taxon sampling could refine species composition of each clade, might reveal additional subclades, and could allow for more rigorous testing of the monophyly of certain infrageneric taxa. In addition, we sought to corroborate hybrid origin in putative hybrid species and to test a hypothesis of higher rates of nucleotide substitution in obligate seeders compared to facultative sprouters.

MATERIALS AND METHODS

Taxon Sampling

The ingroup included a total of 98 accessions representing 83 unique taxa. The remaining 15 accessions were multiple samples of morphologically diverse and/or widely distributed species: *Arctostaphylos uva-ursi*, *A. patula* Greene, *A. pungens* HBK, *A. manzanita* Parry, *A. glandulosa* Eastw., and *A. tomentosa* (Pursh) Lindl. The outgroup contained six species representing the other five genera in the Arbutoideae. All sequences generated for this study were new, except for two accessions of *Arctostaphylos* and five outgroup taxa, which were downloaded from GenBank (Appendix 1). Taxonomy follows Wells (2000) with names, geographic origin, voucher specimen data, and GenBank accession numbers given in Appendix 1. All accessions sequenced for this study are represented by voucher specimens deposited at the Harry D. Thiers Herbarium at San Francisco State University (SFSU), except for *Arctous erythrocarpa*, which is deposited at MO.

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted from herbarium material using DNeasy™ Plant Mini Kits (QIAGEN Corp., Alameda, CA, USA). The nrITS region was amplified using *Arctostaphylos*-specific primers designed for this study (5' to 3'): ITS 4-M = GGAAGTAAAAGTCGTAACAAGG and ITS 5-M = CTGGGGTCGCAATCAAGG. PCR amplifications for the ITS region were carried out under the following conditions: an initial denaturation step (92°C, 2 min) followed by 35 cycles of denaturation (92°C, 30 sec), annealing (58°C, 30 sec), and elongation (72°C, 2 min), with a final extension step (72°C, 5 min). PCR amplifications were performed in 25 µl reactions containing 10–100 ng genomic DNA and 0.25 µl Expand Taq High Fidelity PCR System™ with buffer and Mg^{2+} (Roche Diagnostics, Indianapolis, IN, USA). PCR products were visualized on an agarose gel and purified using MO BIO UltraClean™ PCR Clean-up Kits (MO BIO Laboratories, Carlsbad, CA, USA). Cycle-sequencing reactions used the same primers, annealing temperature, and additive as for PCR and employed BigDye® fluorescent dye-labeled chemistry (BigDye Terminator Cycle Sequencing Kit, Applied Biosystems, Foster City, CA, USA). Cycle-sequenced products were separated and visualized on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA). When a polymorphic nucleotide position was detected, the taxon was sequenced a second time to confirm the polymorphism. DNA sequencing was performed at the San Francisco State University Conservation Genetics Laboratory.

Phylogenetic Analyses

Due to low rates of nucleotide substitution and few insertions/deletions, ITS sequences were easily aligned automatically using Sequencher version 4.1 (Gene Codes, Ann Arbor, MI, USA). Maximum parsimony (MP) analysis was conducted on two data sets: 1) the total data set for all 98 *Arctostaphylos* sequences, and 2) a reduced data set with 17 taxa removed that had polymorphic nucleotide positions. Forty-four ingroup

taxa, which shared identical ITS sequences, were treated as a single "taxon" (the "Gray Leaf" haplotype), therefore the actual number of taxa in both MP analyses was 55 and 38, respectively. MP analyses used the heuristic search strategy of Olmstead and Palmer (1994) to search the tree space for multiple islands of shortest trees. This search strategy, as implemented in PAUP* ver. 4.0b10 (Swofford 2002), was carried out in four steps, with the saved trees from each step used as starting trees in subsequent searches: 1) 1,000 random addition sequence replicates, nearest neighbor interchange (NNI), MulTrees Off, 2) tree-bisection reconnection (TBR), MulTrees Off, 3) NNI, MulTrees On, and 4) TBR, MulTrees On. A strict consensus tree was calculated from the shortest trees saved from each search. Internal branch support of phylogenetic trees was estimated with 100 bootstrap (BS) replicates (Felsenstein 1985) using a full heuristic search with TBR branch swapping, MulTrees on, and 10 random stepwise addition replicates. Results from the BS analysis were summarized in a strict consensus tree. Nucleotide characters were equally weighted and unordered, and indels were treated as missing data.

RESULTS

The aligned sequence matrix for the 98 ingroup accessions and 6 outgroup taxa was 600 bp in length (0% missing data), with 127 variable positions and 62 parsimony informative characters. A one bp deletion at position 403 was detected for *Arctostaphylos malloryi* (W.Knight & Gankin) P.V.Wells, *A. nissenana* Merriam, and three subspecies of *A. viscida* Parry. Forty-four taxa shared an identical nrITS sequence—the "Gray Leaf" haplotype—and are listed in Appendix 2 (the phrase "Gray Leaf haplotype" is used throughout this study to refer to this shared sequence and does not imply a uniform morphology among taxa sharing this identical sequence). The uncorrected pairwise distances for the ingroup nrITS sequences ranged from 0 to 2.4%. Parsimony analysis of the complete ITS data set reached a maximum of 100,000 equally parsimonious trees of length 178 before ending initial tree generation, with a consistency index (CI) of 0.837 (0.753 excluding uninformative characters) and retention index (RI) of 0.881. A strict consensus tree (not shown) revealed a large polytomy and three small clades: 1) a clade of all six accessions of North American *A. uva-ursi* (58% BS), 2) a clade containing *A. pumila* Nutt. and *A. tomentosa* (1) (59% BS), and 3) a clade containing *A. mewukka* and *A. ohloneana* M.C.Vasey & V.T.Parker (90% BS).

Polymorphic nucleotide positions were detected in the sequence electropherograms from superimposed patterns of nucleotide additivity (SNAPs) and were confirmed in 17 taxa (18% of ingroup): *A. otayensis*, *A. pringlei* ssp. *drupacea* Parry (P.V.Wells), *A. mewukka*, *A. pilosula* (Jeps. & Wiesl.), *A. viscida* ssp. *viscida*, *A. manzanita* ssp. *manzanita* from Humboldt Co., CA, *A. manzanita* ssp. *roofii* (Gankin) P.V.Wells, *A. manzanita* ssp. *laevigata* (Eastw.) Munz, *A. manzanita* ssp. *bowermanii*, *A. pungens* from San Diego Co., CA, *A.* ×*pacifica* Roof, *A. nissenana*, *A. edmundsii* J.T.Howell, *A. glandulosa* ssp. *glandulosa* from San Diego Co., CA, *A. myrtifolia*, *A. nummularia* (1) and *A. hispidula* Howell. The number of polymorphic nucleotide positions within an individual ranged from 1 to 6 (0.7% to 4.3% of variable positions). Of these 17 taxa, 12 are tetraploids and/or hybrids. In four putative hybrid-derived or introgressed taxa (*A. pringlei* ssp. *drupacea*, *A. manzanita* ssp. *roofii*, *A. manzanita* ssp. *laevigata*, and *A. hispidula*) sequence electropherograms showed near-perfect patterns of additivity between synapomorphic nucleotides of suspected parents and hybrids (Table 1).

A second MP analysis was conducted with the 17 taxa with polymorphic nucleotide positions removed. The resulting aligned sequence matrix had 126 variable positions with 56 parsimony informative characters. MP analysis of the reduced ITS data set recovered a two-clade topology in the strict consensus of 350 equally parsimonious trees (Fig. 1) (tree length = 169, CI = 0.817, CI excluding uninformative characters = 0.677, RI = 0.858). Clade 1 was weakly supported (55% BS) and Clade 2 was moderately well supported (77% BS). Within Clade 1, two subclades were recovered: 1) a clade containing the six accessions of *A. uva-ursi* (59% BS) and 2) a clade containing three subspecies of *A. stanfordiana* Parry, *A. nummularia* A.Gray, and *A. mendocinoensis* P.V.Wells (76% BS). In Clade 2, the Gray Leaf "taxon" representing the 44 taxa with identical sequences fell out in a polytomy while three clades were resolved: 1) *A. pungens* from Arizona, *A. australis*,

TABLE 1. Four putative hybrid taxa showing superimposed nucleotide additivity patterns (SNAPs) between suspected parents and hybrid offspring. 2*n* = ploidy level (26 = diploid, 52 = tetraploid). Numbers above bases indicate nucleotide position.

Taxon	Relationship	2*n*	Nucleotide position		
			54	363	556
A. glauca	parent	26	G	A	G
A. pringlei subsp. *drupacea*	hybrid	26	G/A	A/G	G/T
A. pringlei subsp. *pringlei*	parent	26	A	G	T
			121	154	
A. patula	parent	52	T	T	
A. manzanita subsp. *roofii*	hybrid	52	C/T	C/T	
A. manzanita subsp. *manzanita*	parent	52	C	C	
			121	154	
A. stanfordiana subsp. *stanfordiana*	parent	26	T	T	
A. manzanita subsp. *laevigata*	hybrid	52	C/T	C/T	
A. manzanita subsp. *manzanita*	parent	52	C	C	
			500		
A. viscida subsp. *viscida*	parent	26	C		
A. hispidula	hybrid	26	C/A		
A. stanfordiana subsp. *stanfordiana*	parent	26	A		

and *A. pringlei* subsp. *pringlei* (71% BS), 2) *A. pumila* and *A. tomentosa* subsp. *tomentosa* (65% BS), and 3) *A. malloryi* and *A. viscida* subsp. *mariposa* (59% BS).

Of the 44 species sharing an identical Gray Leaf haplotype, five are facultative sprouters (*A. glandulosa* ssp. *cushingiana*, *A. glandulosa* ssp. *glaucomollis*, *A. tomentosa* ssp. *daciticola*, *A. tomentosa* ssp. *insulicola*, and *A. tomentosa* ssp. *roseii*) and the rest are obligate seeders. The low variability of the nrITS region—and the fact that both obligate seeders and several sprouters shared an identical sequence—precluded a test of higher nucleotide substitution rates in seeders compared to sprouters.

DISCUSSION

The phylogeny inferred from the nrITS data sets reveals less resolution than had been expected for this morphologically diverse and widely distributed genus. The species composition of Clades 1 and 2 recovered in the MP analysis of the reduced nrITS data set is largely congruent with the two-clade molecular phylogenies of Markos et al. (1998) and Boykin et al. (2005), and the flavonoid phenogram of Denford (1981).

None of the subclades recovered in MP analyses have a discrete pattern of geographical distribution that can be mapped onto the phylogeny. Although the circumarctic distribution of *Arctostaphylos uva-ursi* is unique in the genus, this species is also distributed along the coastal areas and mountains of western North America where it co-occurs with other taxa (e.g., *A. columbiana* Piper, *A. imbricata* Eastw., *A. patula*). Neither are there discrete and readily interpretable morphological or cytological synapomorphies that can be mapped onto this phylogeny to support the present taxonomic groupings of Wells (2000) or the subclades that were recovered in the phylogenetic trees. Boykin et al. (2005) found that the character states of inflorescence bracts, bark, distribution of stomata, and ploidy level were not consistent with monophyly of certain infrageneric groups and that some states have arisen more than once in the genus. Because Wells' (2000) classification of *Arctostaphylos* is based on potentially homoplasious morphological characters, discordance between his classification and the nrITS phylogeny is not unexpected.

The occurrence of polymorphic nucleotide positions showing patterns of additivity between synapomorphic nucleotides of putative hybrids and suspected parents is suggestive of hybrid origin in four taxa (Table 1). However, many more polymorphic sites showing patterns of additivity between parents and hybrid offspring would be needed to confirm hybrid origin for the taxa in Table 1 (e.g., Sang et al. 1995). Such

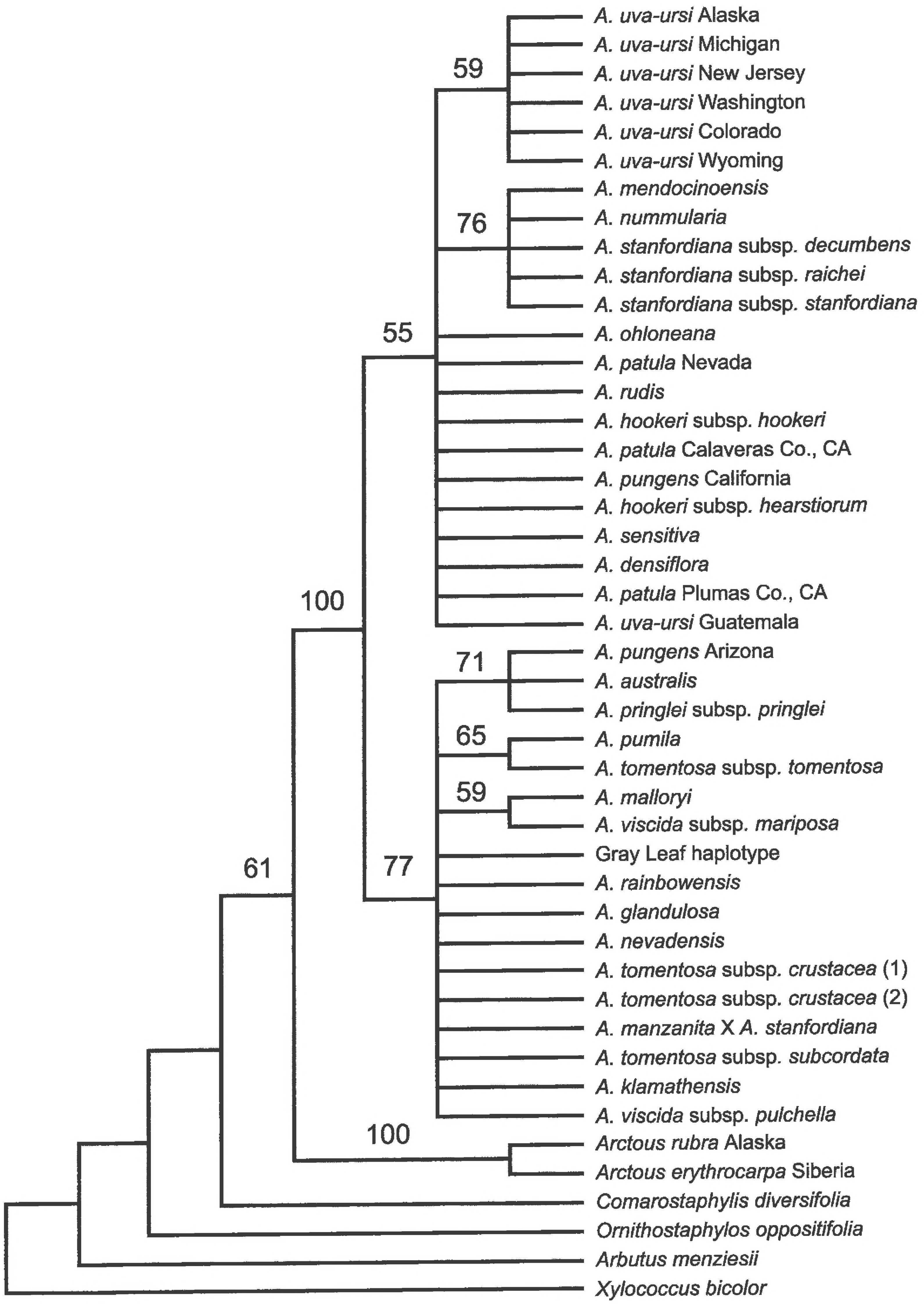

FIG. 1. Strict consensus of 350 most parsimonious trees estimated from nrITS sequences. The numbers above the branches are bootstrap percentages. The taxon "Gray Leaf haplotype" represents 44 taxa with an identical nrITS sequence (listed in Appendix 2).

polymorphisms suggest: 1) recent hybrid origin where both parental alleles are maintained in the hybrid progeny, 2) different alleles existing between parental genomes in tetraploids, 3) recently substituted nucleotides that have not been fully homogenized via concerted evolution, or 4) intra-individual nrITS paralogs (Wendel et al. 1995; Koontz et al. 2004; Bailey et al. 2003).

The 44 taxa sharing the Gray Leaf haplotype are morphologically diverse and are composed of both obligate seeders and facultative sprouters (Appendix 2). Sprouting species sharing the Gray Leaf haplotype are represented by five tetraploid taxa of the *Arctostaphylos glandulosa-tomentosa* complex, whereas most of the other taxa are diploid obligate seeders. As a result, a hypothesis of greater nrITS nucleotide substitution rates in short generation-time seeders vs. long generation-time sprouters could not be tested. These results suggest common ancestry of all "Gray Leaf" taxa, and by extension, the remaining taxa in Clade 2. If phylogenetic reconstruction of *Arctostaphylos* based on nrITS is an accurate inference of common ancestry, then there has been much diversification in the morphology, cytology, and ecological life-histories of taxa in Clade 2 without corresponding nrITS sequence divergence.

Resolving species-level phylogenies is difficult in perennial, reticulating, and putatively recently diversified genera such as *Arctostaphylos* and other genera containing species that are widely distributed and morphologically diverse (e.g., *Ceanothus* (Rhamnaceae), Hardig et al. 2000, *Leucaena* (Fabaceae), Bailey et al. 2004, *Inga* (Fabaceae), Richardson et al. 2001). Low interspecific sequence variability in the nrITS region in *Arctostaphylos* may be due several factors, including recent diversification in California since the end of the Miocene, long generation time between sexual reproduction events, genome homogenization via introgression, and rapid concerted evolution of nuclear ribosomal ITS arrays in both diploids and tetraploids.

The results from this study and others show that direct sequencing of the nrITS region and the chloroplast provide insufficiently variable DNA sequences needed to resolve phylogenetic relationships in *Arctostaphylos*. Other PCR-based techniques have been used in *Arctostaphylos* without success. For example, the presence of endophytic fungi in the sclerophyllous leaves of *Arctostaphylos* prevented the use of RAPDs (randomly amplified polymorphic DNAs) and inter-simple sequence repeats (ISSRs) (C. Reading, pers. comm., Wahlert unpubl. data). Another approach using RFLPs (restriction fragment length polymorphisms) of the chloroplast genome may prove useful in corroborating the hybrid origin of *A. mewukka* (K. Schierenbeck, pers. comm.). Bi-parentally inherited nuclear genes with adequate sequence variability are needed to estimate phylogeny in *Arctostaphylos*. Sequences from single- or low-copy nuclear genes or from anonymous nuclear regions using sequence characterized amplified regions (SCARs) may provide a means to resolve the phylogeny and to tease apart patterns of reticulation in *Arctostaphylos*. Until then, many fascinating questions of polyploidization, hybrid and ecological speciation, biogeography, and ecology will go unanswered in this remarkable genus.

APPENDIX 1

List of taxa used in this study with voucher specimen information and GenBank accession numbers for nrITS sequences. All vouchers are deposited in the Harry D. Thiers Herbarium at San Francisco State University (SFSU), except for *Arctous erythrocarpa*, which is deposited at MO. Numbers in parentheses indicate different accessions of the same taxon. * Denotes a sequence obtained from GenBank.

Outgroup. *Arbutus menziesii* Pursh*, AF086828; *Arctous rubra* (Rehder & E.H.Wilson) Nakai*, AF091944; *Arctous erythrocarpa* Small., Russian Siberia, MO accession number 5196245, GQ281006; *Comarostaphylis diversifolia* (Parry) Greene*, AF091947; *Ornithostaphylos oppositifolia* (Parry) Small*, AF297794 (ITS 1 and 5.8S), AF091962 (5.8S and ITS 2); *Xylococcus bicolor* Nutt.*, AF091948.

Ingroup. *Arctostaphylos andersonii* A.Gray, San Mateo Co., CA, USA, *V.T.Parker & M.C.Vasey 764*, GQ280910; *A. auriculata* Eastw., Contra Costa Co., CA, USA, *M.C.Vasey 840*, GQ280911; *A. australis* Eastw., Baja Norte, Mexico, *M.C.Vasey 799*, GQ280912; *A. bakeri* Eastw. subsp. *bakeri* Eastw., Sonoma Co., CA, USA, *M.C.Vasey 71*, GQ280913; *A. bakeri* subsp. *sublaevis* P.V.Wells, Sonoma Co., CA, USA, *V.T.Parker & M.C.Vasey 548*, GQ280914; *A. canescens* subsp. *canescens* Eastw., Marin Co., CA, USA, *V.T.Parker & M.C.Vasey 443*, GQ280915; *A. canescens* subsp. *sonomensis* (Eastw.) P.V.Wells, Lake Co., CA, USA, *M.C.Vasey 282*, GQ280916; *A. catalinae* P.V.Wells, Santa Barbara Co., CA, USA, *V.T.Parker & M.C.Vasey 155*, GQ280917; *A. columbiana* Piper, Humboldt Co., CA, USA, *M.C.Vasey 1030*, GQ280918; *A. confertiflora* Eastw., Santa Barbara Co., CA, USA, *V.T.Parker & M.C.Vasey 900*, GQ280919; *A. cruzen-*

sis Roof, San Luis Obispo Co., CA, USA, *V.T.Parker et al. 12*, GQ280920; *A. densiflora* M.S.Baker, Sonoma Co., CA, USA, *M.C.Vasey 69*, GQ280921; *A. edmundsii* J.T.Howell, Monterey Co., CA, USA, *M.C.Vasey 819*, GQ280922; *A. gabilanensis* V.T. Parker & M.C.Vasey, San Benito Co., CA, USA, *V.T.Parker & M.C.Vasey 1086*, GQ280923; *A. glandulosa* subsp. *cushingiana* (Eastw.) Adams ex McMinn, Santa Barbara Co, CA, USA, *M.C.Vasey 1002*, GQ280924; *A. glandulosa* subsp. *glandulosa* Eastw. (1), San Diego Co., CA, USA, *M.C.Vasey 724*, GQ280925; *A. glandulosa* subsp. *glandulosa* (2), San Benito Co., CA, USA, *V.T.Parker & M.C.Vasey 456*, GQ280926; *A. glandulosa* subsp. *glaucomollis* P.V.Wells, Los Angeles Co., CA, USA, *M.C.Vasey 1000*, GQ280927; *A. glauca* Lindl., Los Angeles Co., CA, USA, *M.C.Vasey 809*, GQ280928; *A. glutinosa* B.Schreib., Santa Cruz Co., CA, USA, *M.C.Vasey 401*, GQ280929; *A. hispidula* Howell, Del Norte Co., CA, USA, *M.C.Vasey 360*, GQ280930; *A. hookeri* subsp. *franciscana* (Eastw.) Munz, cultivated material; Strybing Arboretum, Golden Gate Park, San Francisco, CA, USA, GQ280931; *A. hookeri* subsp. *hearstiorum* (Hoover & Roof) P.V.Wells*, San Luis Obispo Co., CA, USA, AF106817; *A. hookeri* subsp. *hookeri* G.Don, Santa Cruz Co., CA, USA, *V.T.Parker & M.C.Vasey 55*, GQ280932; *A. hookeri* subsp. *montana* (Eastw.) P.V.Wells, Marin Co., CA, USA, *S. Markos 613*, GQ280933; *A. hookeri* subsp. *ravenii* P.V.Wells*, San Francisco Co., CA, USA, AF106819; *A. hooveri* P.V.Wells, Monterey Co., CA, USA, *M.C.Vasey 1050*, GQ280934; *A. imbricata* Eastw., San Mateo Co., CA, USA, *M.C.Vasey 18*, GQ280935; *A. insularis* Greene, Santa Barbara Co., CA, USA, *Anonymous s.n.*, GQ280936; *A. klamathensis* S.W.Edwards, Keeler-Wolf & W.Knight, Siskiyou Co., CA, USA, *M.C.Vasey 756*, GQ280937; *A. luciana* P.V.Wells, San Luis Obispo Co., CA, USA, *M.C.Vasey 1046*, GQ280938; *A. malloryi* (W.Knight & Gankin) P.V.Wells, Shasta Co., CA, USA, *V.T.Parker & M.C.Vasey 834*, GQ280939; *A. manzanita* subsp. *bowermanii* Roof, Contra Costa Co., CA, USA, *M.C.Vasey 169*, GQ280940; *A. manzanita* subsp. *elegans* (Jeps.) P.V.Wells, Glenn Co., CA, USA, *M.C.Vasey 216*, GQ280941; *A. manzanita* subsp. *glaucescens* P.V.Wells, Sonoma Co., CA, USA, *V.T.Parker & M.C.Vasey 508*, GQ280942; *A. manzanita* subsp. *laevigata* (Eastw.) Munz, Contra Costa Co., CA, USA, *M.C.Vasey 894*, GQ280943; *A. manzanita* subsp. *manzanita* Parry (1), Mendocino Co., CA, USA, *V.T.Parker & M.C.Vasey 369*, GQ280944; *A. manzanita* subsp. *manzanita* (2), Humboldt Co., CA, USA, *M.C.Vasey 851*, GQ280945; *A. manzanita* subsp. *manzanita* (3), Amador Co., CA, USA, *M.C.Vasey 767*, GQ280946; *A. manzanita* subsp. *manzanita* (4), Sonoma Co., CA, USA, *M.C.Vasey 424*, GQ280947; *A. manzanita* subsp. *roofii* (Gankin) P.V.Wells, Glenn Co., CA, USA, *M.C.Vasey 883*, GQ280948; *A. manzanita* × *A. stanfordiana*, Sonoma Co., CA, USA, *M.C.Vasey 425*, GQ280949; *A.* × *media* Greene, Mendocino Co., CA, USA, *M.C.Vasey 253*, GQ280950; *A. mendocinoensis* P.V.Wells, Mendocino Co., CA, USA, *G.Wahlert s.n.*, GQ280951; *A. mewukka* Merriam, Tuolumne Co., CA, USA, *M.C.Vasey 575*, GQ280952; *A. montaraensis* Roof, San Mateo Co., CA, USA, *M.C.Vasey 743*, GQ280953; *A. montereyensis* Hoover, Monterey Co., CA, USA, *M.C.Vasey 656*, GQ280954; *A. morroensis* Wiesl. & B. Schreib., San Luis Obispo Co., CA, USA, *K. Bode 823*, GQ280955; *A. myrtifolia* Parry, Amador Co., CA, USA, *M.C.Vasey 733*, GQ280956; *A. nevadensis* A.Gray, Siskiyou Co., CA, USA, *M.C.Vasey 757*, GQ280957; *A. nissenana* Merriam, Placer Co., CA, USA, *M.C.Vasey 892*, GQ280958; *A. nummularia* A.Gray, Mendocino Co., CA, USA, *V.T.Parker & M.C.Vasey 516*, GQ280959; *A. obispoensis* Eastw., Monterey Co., CA, USA, *M.C.Vasey 1052*, GQ280960; *A. ohloneana* M.C.Vasey & V.T.Parker, Santa Cruz Co., CA, USA, *V.T.Parker & M.C.Vasey 111*, GQ280961; *A. osoensis* P.V.Wells, San Luis Obispo Co., CA, USA, *K. Bode 780*, GQ280962; *A. otayensis* Wiesl. & Schreib., San Diego Co., CA, USA, *M.C.Vasey 748*, GQ280963; *A.* × *pacifica* Roof, San Mateo Co., CA, USA, *M.C.Vasey 20*, GQ280964; *A. pajaroensis* J.E.Adams, Monterey Co., CA, USA, *V.T.Parker & M.C.Vasey 459*, GQ280965; *A. pallida* Eastw., Alameda Co., CA, USA, *M.C.Vasey 436*, GQ280966; *A. patula* Greene (1), White Pine, Co., Nevada, USA, *M.C.Vasey 626*, GQ280967; *A. patula* (2), Plumas Co., CA, USA, *M. Wood 316*, GQ280968; *A. patula* (3), Calaveras Co., CA, USA, *V.T.Parker & M.C.Vasey 770*, GQ280969; *A. pilosula* Jeps. & Wiesl., San Luis Obispo Co., CA, USA, *M.C.Vasey 233*, GQ280970; *A. pringlei* subsp. *drupacea* Parry, Riverside Co., CA, USA, *M.C.Vasey 993*, GQ280971; *A. pringlei* subsp. *pringlei* Parry, Pima Co., Arizona, USA, *M.C.Vasey 232*, GQ280972; *A. pumila* Nutt., Monterey Co., CA, USA, *V.T.Parker & M.C.Vasey 677*, GQ280973; *A. pungens* HBK (1), San Bernardino Co., CA, USA, *M.C.Vasey 194*, GQ280974; *A. pungens* (2), San Diego Co., CA, USA, *M.C.Vasey 696*, GQ280975; *A. pungens* (3), Pima Co., Arizona, USA, *M.C.Vasey 228*, GQ280976; *A. purissima* P.V.Wells, Santa Barbara Co., CA, USA, *V.T.Parker et al. 2*, GQ280977; *A. rainbowensis* J.E.Keeley & Massihi, San Diego Co., CA, USA, *K. Bode 775*, GQ280978; *A. regismontana* Eastw., San Mateo Co., CA, USA, *M.C.Vasey 557*, GQ280979; *A.* × *repens* J.T.Howell, Marin Co., CA, USA, *V.T.Parker & M.C.Vasey 276*, GQ280980; *A. rudis* Jeps. & Wiesl., San Luis Obispo Co., CA, USA, *V.T.Parker & M.C.Vasey 151*, GQ280981; *A. sensitiva* Jeps., Marin Co., CA, USA, *V.T.Parker 306*, GQ280982; *A. silvicola* Jeps. & Wiesl., Santa Cruz Co., CA, USA, *V.T.Parker & M.C.Vasey 865*, GQ280983; *A. stanfordiana* Parry subsp. *decumbens* (P.V.Wells) P.V.Wells, Sonoma Co., CA, USA, *M.C.Vasey 422*, GQ280984; *A. stanfordiana* Parry subsp. *raichei* W.Knight, Mendocino Co., CA, USA, *M.C.Vasey 277*, GQ280985; *A. stanfordiana* Parry subsp. *stanfordiana*, Napa Co., CA, USA, *M.C.Vasey 392*, GQ280986; *A. tomentosa* subsp. *crustacea* (Eastw.) P.V.Wells (1), Monterey Co., CA, USA, *V.T.Parker 584*, GQ280987; *A. tomentosa* subsp. *crustacea* (2), Santa Cruz Co., CA, USA, *M.C.Vasey 786*, GQ280988; *A. tomentosa* subsp. *daciticola* P.V.Wells, San Luis Obispo Co., CA, USA, *M.C.Vasey 1008*, GQ280989; *A. tomentosa* subsp. *insulicola* P.V.Wells, Santa Barbara Co., CA, USA, *V.T.Parker & M.C.Vasey 903*, GQ280990; *A. tomentosa* subsp. *rosei* (Eastw.) P.V.Wells, Monterey Co., CA, USA, *M.C.Vasey 822*, GQ280991; *A. tomentosa* subsp. *subcordata* (Eastw.) P.V.Wells, Santa Barbara Co., CA, USA, *V.T.Parker & M.C.Vasey 901*, GQ280992; *A. tomentosa* subsp. *tomentosa* Pursh, Monterey Co., CA, USA, *M.C.Vasey 243*, GQ280993; *A. uva-ursi* (L.) Spreng., Ocean Co., New Jersey, USA, *V.T.Parker 437*, GQ280994; *A. uva-ursi*, Sleeping Bear National Lakeshore, Michigan, USA, *McEaghan 933*, GQ280995; *A. uva-ursi*, Yellowstone National Park, Wyoming, USA, *V.T.Parker & M.C.Vasey 942*, GQ280996; *A. uva-ursi*, Gunnison Co., Colorado, USA, *M.C.Vasey 643*, GQ280997; *A. uva-ursi*, Alaska, USA, *J. Bishop 684*, GQ280998; *A. uva-ursi*, Clallam Co., Washington, USA, *M.C.Vasey 340*, GQ280999; *A. uva-ursi* subsp. *cratericola* (J.D.Smith) P.V.Wells, cultivated material from Guatemala, U.C. Botanical Garden, 92-0545, GQ281000; *A. virgata* Eastw., Marin Co., CA, USA, *M.C.Vasey 783*, GQ281001; *A. viscida* subsp. *mariposa* (Dudley) P.V.Wells, Tuolumne Co., CA, USA, *M.C.Vasey 499*, GQ281002; *A. viscida* subsp. *pulchella* (Howell) P.V.Wells, Sonoma Co., CA, USA, *M.C.Vasey*

470, GQ281003; *A. viscida* subsp. *viscida* Parry, Eldorado Co., CA, USA, *V.T.Parker & M.C.Vasey 849*, GQ281004; *A. wellsii* W.Knight, San Luis Obispo Co., CA, USA, *M.C.Vasey 1041*, GQ281005.

APPENDIX 2

Forty-four taxa with identical nrITS sequences (i.e., the "Gray Leaf" haplotype).

Arctostaphylos andersonii, A. auriculata, A. bakeri ssp. *bakeri, A. bakeri* ssp. *sublaevis, A. canescens* ssp. *canescens, A. canescens* ssp. *sonomensis, A. catalinae, A. columbiana, A. confertiflora, A. cruzensis, A. gabilanensis, A. glandulosa* ssp. *cushingiana, A. glandulosa* ssp. *glaucomollis, A. glauca, A. glutinosa, A. hookeri* ssp. *franciscana, A. hookeri* ssp. *montana, A. hookeri* ssp. *ravenii, A. hooveri, A. imbricata, A. insularis, A. luciana, A. manzanita* ssp. *elegans, A. manzanita* ssp. *glaucescens, A. manzanita* ssp. *manzanita (1), A. manzanita* ssp. *manzanita (3), A. manzanita* ssp. *manzanita (4), A. × media, A. montaraensis, A. montereyensis, A. morroensis, A. obispoensis, A. osoensis, A. pajaroensis, A. pallida, A. purissima, A. regismontana, A. × repens, A. silvicola, A. tomentosa* ssp. *daciticola, A. tomentosa* ssp. *insulicola, A. tomentosa* ssp. *roseii, A. virgata, A. wellsii.*

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